Arch Irn Med 2002; 5 (1): 44-47

ORIGINAL ARTICLE

ANTICONVULSANT EFFECTS OF AQUEOUS AND ETHANOLIC EXTRACTS OF *CROCUS SATIVUS* L. STIGMAS IN MICE

Hossein Hosseinzadeh PhD•, Vahid Khosravan

Faculty of Pharmacy, Mashad University of Medical Sciences, Mashad, Iran

Abstract

Background-*Crocus sativus* L. stigma (CSS) has sedative properties and is used in traditional medicine for its anticonvulsant property.

Objective-We studied the anticonvulsant activity of the aqueous and ethanolic extracts of CSS in mice in order to evaluate the traditional use of this plant.

Methods-The pentylenetetrazole (PTZ) and the maximal electroshock seizure (MES) tests were used for assessing the anticonvulsive effects of this plant.

Results-In the PTZ test, CSS delayed the onset of tonic convulsions, but failed to produce complete protection against mortality. In the MES test, both extracts decreased the duration of tonic seizures.

Conclusion-The results of this study indicate that the extracts of CSS may be beneficial in both absence and tonic clonic seizures.

Keywords • *Crocus sativus* L. stigmas • anticonvulsant activity • herbal medicine

Introduction

The stigma of the plant *Crocus sativus* L., commonly known as saffron is used in traditional medicine as an aphrodisiac, antispasmodic and expectorant.[1] Recent pharmacological studies have demonstrated that saffron extract has antitumor[2-4] and hypolipidemic effects[5] as well as radical scavengering and learning or memory-improving properties.[5-6]

Chemical studies have shown that *C. sativus* contains constituents such as crocin, crocetin safranal and picrocrocin.[7-9] Among the constituents of saffron extract, crocetin is mainly responsible for the above pharmacological activities.[5]

In traditional medicine, the stigmas of this plant have been used as an anticonvulsant remedy.[1] The aim of this study was to evaluate the anticonvulsant effect of *C. sativus* stigma by the maximal electroshock seizure (MES) and pentylenetetrazole (PTZ) tests.

•**Correspondence:** *H. Hosseinzadeh PhD, Faculty of Pharmacy, Mashad University of Medical Sciences, Mashhad, Iran. P.O.Box: 91775-1365, Fax: +98-511-8437075, E-mail: hosseinzadehh@yahoo.com.*

Materials and Methods

Animals

Male and female albino mice, weighing 25 to30 g, were obtained from a randomly-bred colony and maintained on a special diet (Khorassan Javaneh Co, Mashad, Iran). They were kept in the animal house of Mashad University of Medical Sciences, in colony rooms with 12/12 h light/dark cycle at 21±2˚ C. The animals had free access to food and water. The mice were divided into 4 groups as follows: 144 mice in the PTZ seizure test group, 144 mice in the MES group, 96 mice in the toxicity and 20 mice in the best time of treatment group.

Plant material

Stigmas were collected from the suburbs of Ghaen City (North Iran) in October 1998, dried in the shadow, and subsequently grounded. The *C. sativus* L. plants were identified by Ferdowsi University (Ms Molaei) and voucher samples were preserved for reference in the herbarium of the Faculty of Pharmacy of Mashhad Universityof Medical Sciences. The specimen number of the plant is 134-0319-1.

Table 1. Effect of aqueous extract of *Crocus sativus* stigmas on the onset of seizure of pentylenetetrazole-induced convulsion and death in mice.

Treatment	Dose	Onset of seizure (sec)	Mortality protection (%)
Normal saline	20 mL/kg	43.9±0.9	0
Phenobarbital	10 mg/kg	90.0±1.7*	0
	20 mg/kg	172.3±3.3*	37.5
	30 mg/kg	296.5±3.9*	62.5
	40 mg/kg	372.9±2.4*	100
Aqueous extract	0.08 g/kg	86.5±4.2*	0
	0.32 g/kg	135.32±1.6*	0
	0.56 g/kg	300.3±0.8*	25
	0.80 g/kg	267.3±3.8*	25

The extracts and phenobarbital were injected intraperitoneally 20 and 45 min before the administration of pentylenetetrazole (IP, 90 mg/kg), respectively. Values are presented as mean±SEM for the duration of tonic seizure for 8 mice; *p<0.001, compared to normal saline using Tukey-Kramer test.

Preparation of extracts

In the maceration method, 3g of stigma was macerated in 400 mL ethanol (80%, v/v) (yield: 62.43) or distilled water (yield: 60.67) for three days. The mixture was subsequently filtered and concentrated under reduced pressure at 35˚ C.

Anticonvulsant activity

***Pentylenetetrazole (PTZ) seizure test*[10]**

A total of 144 mice were divided into 8 groups. The aqueous and ethanolic extracts as well as phenobarbital (Merck, Germany) were injected intraperitoneally 20 and 45 min before administration of 90mg/kg pentylenetetrazole (Aldrich, Germany), respectively. Time before onset of clonic convulsions and the percentage of mortality were recorded.

Maximal electroshock seizure (MES) test[10]

A total of 144 mice were divided into 8 groups. An alternating current of 50 Hz and 150 mA was delivered to experimental animals through bicorneal electrodes for 0.2 sec. A drop of 0.9% saline solution was poured into both eyes prior to placing the electrodes. The aqueous and ethanolic extracts as well as phenobarbital were injected intraperitoneally 45 min before induction of MES. Duration of tonic convulsions (a hindlimb tonic extension) and the percentage of seizure protection and mortality were recorded.

The maximum non-fatal dose and acute toxicity

A total of 96 mice were divided into 4 groups and different doses of the extracts were injected to them. After 48 h, the maximum dose that had not induced mortality, was considered as the maximum non-fatal dose. LD50 values and the corresponding confidence intervals were determined by the Litchfield and Wilcoxon methods (PHARM/PCS Version 4, USA).

Data were expressed as mean values±SEM and tested with ANOVA and Tukey-Kramer tests.

Results

The maximum non-fatal doses of the aqueous and ethanolic extracts were 0.8 and 2 g/kg,

Table 2. Effect of ethanolic extract of *Crocus sativus* stigma on the onset of seizure of pentylenetetrazole-induced convulsion and mortality in mice.

Treatment	Dose	Onset of seizure (sec)	Mortality protection (%)
Normal saline	20 mL/kg	58.5±3.7	0
Phenobarbital	10 mg/kg	87.8±2.7*	0
	20 mg/kg	178.3±2.9*	25
	30 mg/kg	308.5±3.8*	75
	40 mg/kg	379.1±3.7*	100
Ethanolic extract	0.2 g/kg	95.9±3.0*	0
	0.8 g/kg	118.1±3.4*	0
	1.4 g/kg	147.6±3.7*	12.5
	2.0 g/kg	244.4±5.3*	25

The extracts and phenobarbital were injected intraperitoneally 20 and 45 min before the administration of pentylenetetrazole (i.p., 90 mg/kg), respectively. Values presented as the mean±SEM duration of tonic seizure for 8 mice; *p<0.001, compared to normal saline using Tukey-Kramer test.

Table 3. Effect of aqueous extract of *Crocus sativus* stigma on the duration and inhibition of seizure and mortality in maximal electroshock-induced seizure in mice.

Treatment	Dose	Duration of tonic seizure (sec)	Seizure protection(%)	Mortality protection(%)
Normal saline	20 mL/kg	27.8±1.0	0	87.5
Phenobarbital	10 mg/kg	18.0±0.6*	25	87.5
	20 mg/kg	14.4±0.4*	37.5	100
	30 mg/kg	9.6±0.5*	62.5	100
	40 mg/kg	5.8±0.5*	75	100
Aqueous extract	0.08 g/kg	17.1±0.7*	0	75
	0.32 g/kg	17.0±1.4*	0	87.5
	0.56 g/kg	12.3±0.3*	0	100
	0.80 g/kg	10.5±0.5*	0	100

The extracts and phenobarbital were injected intraperitoneally 20 and 45 min before induction of maximal electroshock seizures, respectively. Values presented as the mean±SEM duration of tonic seizure for 8 mice; *p<0.001, compared to the normal saline using Tukey-Kramer test.

respectively. LD50 values of aqueous and ethanolic extracts were 1.6 g/kg (95% CI: 1.2-2.2) and 3.4 g/kg (95% CI: 2.5-4.5), respectively.

Both ethanolic and aqueous extracts increased the latency of convulsions induced by PTZ dose-dependently, but failed to produce complete protection against mortality (Tables 1 and 2). At the dose of 80 mg/kg, the aqueous extract had an efficacy similar to 10 mg/kg of phenobarbital used in the PTZ test.

In the MES test, the aqueous (0.8 g/kg) and ethanolic (2 g/kg) extracts as well as phenobarbital (40 mg/kg) decreased the duration of tonic seizures by 62%, 49% and 79%, respectively (Tables 3 and 4). Neither extracts had seizure-protective effects in the MES test.

Discussion

The present study indicates that the aqueous and ethanolic extracts of *C. sativus* have anticonvulsant activity in PTZ and MES-induced seizures.

Compared with a toxicity classification[11], the extracts are relatively toxic. The ethanolic extract was found to be more toxic than the aqueous extract.

Agents affecting the PTZ test can inhibit absence seizures.[10] Thus *C. sativus* may have some beneficial effect on this kind of seizure in clinical trials. The extracts showed activity against maximal electroshock seizures. This implies that the extracts can improve tonic clonic seizures.[10] The mechanism (s) of anticonvulsant activity of the extracts is not clear. Saffron has been reported to have some behavioral effects on the central nervous system. In one study an alcoholic extract of *C. sativus* decreased the motor activity and prolonged the sleeping time induced by hexobarbital.[6] This study suggests that the

Table 4. The effect of ethanolic extracts of *C. sativus* stigmas on the duration, inhibition of seizure and mortality protection against seizure induced by maximal electroshock in mice.

Treatment	Dose	Duration of tonic seizure (sec)	Seizure protection percent	Mortality protection percent
Normal saline	20 mL/kg	28.1±0.30	0	87.5
Phenobarbital	10 mg/kg	16.3±0.3*	25	87.5
	20 mg/kg	12.5±0.5*	25	100
	30 mg/kg	9.6±0.5*	75	100
	40 mg/kg	5.9±0.3*	75	100
Ethanolic extract	0.2 g/kg	22.8±0.5*	0	75
	0.8 g/kg	19.0±0.3*	0	75
	1.4 g/kg	17.5±1.1*	0	87.5
	2.0 g/kg	14.4±0.5*	0	100

The extracts and phenobarbital were injected intraperitoneally 20 and 45 min before induction of maximal electroshock seizures, respectively. Values presented as the mean±SEM duration of tonic seizure for 8 mice; *p<0.001, compared to the normal saline using Tukey-Kramer test.

ethanolic extract possesses a sedative effect, which is probably responsible for the anticonvulsant effect of the extracts. In another study the plant was found to increase learning and memory performance in experimental animals.[5]

We conclude that the aqueous and ethanolic extracts of CSS have anticonvulsant activity in PTZ and MES tests that may help control petit mal and grand mal seizures.

References

1 Zargari A. *Medicinal Plants.* Tehran: Tehran University Press; 1990: 574-8.

2 Nair SC, Pannikar B, Panikkar KR. Anti-tumor activity of saffron (*Crocus sativus*). *Cancer Lett.* 1991; **57:** 109-14.

3 Salomi MJ, Nair SC, Panikkar KR. Inhibitory effects of *Nigella sativus* and saffron (*Crocus sativus*) on chemical carcinogenesis in mice. *Nutr Cancer*. 1991; **16:** 67-72.

4 Nair SC, Kurumboor SK, Hasegawa JH. Saffron chemoprevention in biology and medicine: a review. *Cancer Biother*. 1995; **10:** 257-64.

5 Abe K, Saito H. Effects of saffron extract and its constituent crocin on learning behavior and long-term potentiation. *Phytother Res*. 2000; **14:** 149-52.

6 Zhang Y, Shoyama Y, Sugiura M, Saito H. Effect of *Crocus sativus* L. on the ethanol-induced impairment of passive avoidance performances in mice. *Biol Pharm Bull.* 1994; **17:** 217-21.

7 Tarantilis PA, Tsoupras G, Polissiou M. Determination of saffron (*Crocus sativus* L.) components in crude plant extract using high-performance liquid chromatography-UV-visible photodiode-array detection-mass spectrometry. *J Chromatogr A*. 1995; **699:** 107-18.

8 Escribano J, Alonso GL, Coca-Prados M, Fernandez JA. Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells *in vitro*. *Cancer Lett.* 1996; **100:** 23-30.

9 Lozano P, Delgado D, Gomez D, Rubio M, Iborra JL. A non-destructive method to determine the safranal content of saffron (*Crocus sativus* L.) by supercritical carbon dioxide extraction combined with high-performance liquid chromatography and gas chromatography. *J Biochem Biophys Method.* 2000; **43:** 367-8.

10 Vida JA. Anticonvulsants. In: Foye WO, Lemke TL, Williams DA, eds. *Principles of Medicinal Chemistry*. London: Williams and Wilkins; 1995.

11 Loomis TA. *Essentials of Toxicology*. Philadelphia: Lea and Febiger; 1968: 67-78.